CAN A SINGLE SPERMATOZOÖN INITIATE DEVELOPMENT IN ARBACIA?[1]

OTTO GLASER.

During the summer of 1913 while making the camera lucida tracings on which I have based my comparisons between the volumes of the unfertilized and fertilized ova of *Arbacia*,[2] it became necessary, in order to prevent rotation on the part of the eggs, and the consequent necessity of readjusting the focus, to employ very attenuated suspensions of sperm. The result of the highest dilutions used in these experiments, however, gave an unforeseen result since the appearance of the fertilization membranes was either very much delayed, or failed entirely to take place. This observation suggested the idea of a mass effect of the spermatozoa, and the possibility that this might play a rôle in normal fertilization.

At that time I had already made observations which had convinced me that the fertilization membrane in this egg is not formed de novo, but is preformed in the unfertilized egg, and simply rendered visible by changes occurring at the time of impregnation.[3]

The mechanism through which the fertilization membrane becomes visible will be dealt with in detail at another time; for the present it is sufficient to say that the absorption of water plays an important rôle. It occurred to me therefore that the prevention of this absorption and perhaps the prevention of fertilization itself might be possible even with the employment of more concentrated suspensions of sperm, if the eggs were first treated with Ca. As a matter of fact, it was either difficult or impossible to fertilize eggs so treated. The spermatozoa were active enough, but failed to enter, and fertilization membranes did not appear The following protocol is typical: In a small watch

[1] From the Marine Biological Laboratory at Woods Hole, and the Zoölogical Laboratory of the University of Michigan.

[2] "The Change in Volume of *Arbacia* and *Asterias* Eggs at Fertilization," BIOLOGICAL BULLETIN, Vol. XXVI, pp. 84–91.

[3] "On Inducing Development in the Sea-Urchin (*Arbacia punctulata*), together with Considerations on the Initiatory Effect of Fertilization," *Science*, Vol. XXXVIII., pp. 446–450.

crystal, 4 volumes of fairly dense egg-suspension in sea-water+2 volumes n $CaCl_2$. After two minutes washed in sea-water.

12.26 insemination moderate.
12.28 0 fertilization membrane.
12.30 1 " "
12.34 1 " "

Control normal. All eggs with fertilization membranes in 3–5 minutes after insemination. 100 per cent. cleavage. Hundreds of eggs examined in both control and experiment. In Ca-eggs very few divisions.

In connection with these experiments I noticed that insemination with great excesses of sperm frequently led to results at variance with the above, for fertilization membranes appeared about the majority of the eggs despite the use of Ca, and these eggs developed. This experience strengthened my belief, not only in the validity of the Ca-experiments, but also in the correctness of the original idea, namely that the number of spermatozoa that come into contact with the egg may make a difference.

Encouraged by this result, I diluted a sperm-suspension until only the faintest trace of opalescence remained. Several drops of this attenuated fluid were then drawn up into a medicine dropper of medium size and expelled quantitatively. If the dropper, which of course remained infected with sperm, was then used to agitate eggs in a small quantity of sea-water by carefully drawing the water in and expelling it several times, it was found that very soon a few spermatozoa had attached themselves to every egg. In an optical diameter, 4 to 5 sperm could easily be distinguished, but I awaited further changes in vain, despite the fact that the spermatozoa seemed to have reached the eggs, exhibited the usual amount of activity, and were potent in 100 per cent. of the cases when applied in larger quantities to eggs of the same lot. The following experiment is illustrative:

12.17 insemination with infected pipette.
12.18 0 fertilization membranes.
12.19 0 " "
12.20 1 " "
12.21 2 " "
12.22 3 " "
12.30 3 " "

Control normal; all eggs with fertilization membranes in 3 to 5 minutes. Experimental eggs examined at irregular intervals throughout the day, but no increase in the number of membranes.

Whether the appearance of a fertilization membrane, and impregnation itself will fail to take place in other eggs under similar conditions cannot be predicted, and is perhaps even improbable. With the eggs of *Arbacia punctulata* however I repeated these tests so often that I cannot doubt the correctness of my observations, and I therefore fail to understand Kite's[1] claim that he succeeded in calling forth a fertilization membrane in this egg by means of a single spermatozoön. I imagine that his method involved factors whose importance was unsuspected, since he says: "The real difficulty with this type of experiment is not the size of the spermatozoön, but the fact that when four or five are injected into the egg-jelly, they usually swim out and away from the egg. This necessitates the making of many injections in order to get a single spermatozoön to attach itself to the vitelline membrane and start the reaction." The "making of many injections" very likely involves touching the vitelline membrane an equal number of times, which recalls an experiment mentioned in my earlier paper[2] in which fertilization membranes were induced by surrounding the eggs with large numbers of minute infusoria. Observation indicated a continuous bombardment of the ova.

A quantitative relation between the rate of appearance of the membrane and the agencies, spermatozoa, normally calling it forth is really no more surprising than the efficacy of Ca as an inhibitor. Since now sea-water of sufficient hypotonicity will of itself call forth membranes[3] one may expect the exact reverse of the Ca-experiments if one immerses the eggs briefly in hypotonic solutions. Such ova, if not submerged too long so that the

[1] G. L. Kite, "The Nature of the Fertilization Membrane of the Egg of the Sea Urchin (*Arbacia punctulata*)," *Science*, Vol. XXXVI., pp. 562–564.

[2] *Science, loc. cit.*

[3] In my preliminary communication (*Science, loc. cit.*) I considered the method of "inducing" a fertilization membrane in *Arbacia* by means of hypotonic sea-water new. Schücking however described this procedure in the year 1903. (*Arch. f. d. ges. Physiol.*, Vol. 97, p. 85.) The same method was used on *Arbacia* eggs by McClendon in 1910. (*American Journ. Physiol.*, p. 246.)

appearance of the membrane would have to be attributed to the hypotonic treatment itself, should be capable of fertilization by means of the sperm-infected medicine dropper. Actually under these circumstances fertilization with only 4 to 5 spermatozoa visible in the optical equator is possible in a considerable number of eggs.

PROTOCOL.

In a watch crystal 3 volumes of sea-water+3 volumes of distilled. Added 1 volume of an egg-suspension in normal sea-water. At the instant when the first indications of membrane "initiation" were noticeable added 3 volumes of "double sea-water," *i. e.*, sea-water whose volume had been reduced one-half by boiling. By means of a sperm-infected pipette every egg was provided with 4 to 5 spermatozoa. In a series of microscopic fields the number of undivided eggs was later compared with the number that had divided. The results were:

Experiment I.		Experiment II.	
Undivided.	Divided.	Undivided	Divided.
7	2	4	1
4	3	3	2
4	4	12	2
7	2	18	0
1	2	6	5
6	0	6	0
2	4	7	4
5	1	8	0
5	4	8	5
5	2	14	4
3	2	14	3
5	3	12	3
5	3	13	1
6	5	8	2
2	6	6	3
4	3	7	1
		13	2
		6	1
		6	3
		10	1
		8	2
Total...71	46	189	45
Per cent.61	39	81	19

Controls: Normal eggs+usual amount of sperm = 100% Fertilization. Eggs treated as above+usual amount of sperm = 100% Fertilization. This is in sharp contrast with the earlier experiments in which the operations were carried out at the same

dilutions but without the brief fore-treatment with hypotonic sea-water. Results which harmonize with these but prove less satisfactory on account of injuries to the eggs can be gotten by the use of heat. In this case one might think of a parthenogenetic effect, but in *Arbacia* at least, it is not easy to confuse the usual parthenogenetic cleavage with normal two or four-cell stages.

It is very easy to misunderstand these experiments and to draw wrong conclusions. There is no more doubt in *Arbacia punctulata* than in any other form that a single spermatozoön is sufficient to carry out the biparental effect. Furthermore the experiments with dilute sperm do not in anyway enable us to prejudge what would happen in another egg under similar conditions nor do they warrant the inference that the initiation of development by a single sperm is impossible in *Arbacia* ova deprived of their superficial coverings. I feel very sure of this however: In *Arbacia* the appearance of the fertilization membrane after insemination is a sign that the egg investments have allowed the sperm to pass through. This passage has been possible because the coverings have changed. The change depends on a synchronous softening and absorption of water, the latter having consequences as the result of which the membrane becomes visible. Inasmuch as the becoming visible of the membrane is a reliable index of fertilization, and one of the consequences of fertilization is the division of the ovum, we may say that the initiation of development by a single spermatozoön in this case is impossible because a single sperm cannot effect those changes in the egg-coverings which will permit it to reach the protoplasmic surface film that lies beneath. The situation is exactly as though the entrance to a room were blocked by a barrier which a single man could not break down, although a group of ten might. Once broken down, any one of the men could cross the threshold, but for the opportunity of doing this, the services of the others would be needed. With this analogy in mind, the statement that a single spermatozoön cannot except possibly under special conditions, fertilize the normally invested egg of *Arbacia punctulata*, would appear to agree with the facts.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF MICHIGAN,
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